ABSTRACT

- A calibration procedure is presented for the quantification of melamine in spiked milk samples, based on the application of Weighted Total Least-Squares (WTLS), together with the evaluation of the associated uncertainty, carried out by applying the Law of Propagation of Uncertainty (LPU).
- This approach was applied to an experimental innovative method developed and validated at INRIM based on the Surface Enhanced Raman Scattering (SERS).
- SERS was exploited for the screening and the quantification of melamine in milk due to its specific fingerprint in the Raman spectrum and to the huge enhancement of the melamine Raman signals promoted by its interaction with gold nanoparticles (AuNPs).

2. QUANTIFICATION OF MELAMINE BY RAYNER SERS

- The INRIM method in fig. 2 [1] was developed in accordance with the European law limits for melamine in foodstuffs [2].
- Samples: Aliquots of a 100 mg L⁻¹ melamine solution were added to the semi-skimmed milk. After the extraction procedure, which results in a 10-fold dilution of the samples, the amount of substance concentrations of melamine in the milk samples are 0.099 mg L⁻¹ and 0.291 mg L⁻¹.
- Calibration: The Raman spectrophotometer was calibrated by means of melamine standard solutions in a non-spiked milk extract, in the concentration range (0.015-0.50) mg L⁻¹. The software used for determining the calibration curve of the Raman spectrophotometer is the Calibration Curves Computing (CCS) software [3], developed at INRIM.

4. UNCERTAINTY EVALUATION

- For the evaluation of \( \delta(C_{\text{calc}}) \), the inverse of the calibration curve \( y = a + bx \), i.e. the analysis curve, obtained via WTLS regression [4] was used. The analysis curve (eq. 3) gives the values of the unknown amount of substance concentration of melamine \( C_{\text{calc}} \) in each spiked extract analysed by SERS:

\[
C_{\text{calc}} = \frac{(y - a)}{b}
\]  

(3)

- The theoretical amount of substance concentration of melamine \( C_{\text{theor}} \) in each spiked extract was obtained by a dilution process. The uncertainty \( \delta(C_{\text{theor}}) \) was evaluated by applying the LPU to the model equation (eq. 4):

\[
C_{\text{theor}} = C_i \frac{V_L}{(V_i + V_L)}
\]  

(4)

where:

- \( C_i \) = initial amount of substance concentration of the melamine solution to be added to the milk sample (in mg L⁻¹)
- \( V_L \) = initial volume of the melamine solution (in L)
- \( V_i \) = volume of the dilution solvent (in L)

- The main advantage of the WTLS is the possibility to deal with regression problems involving uncertain and correlated input and output variables - e.g. the determination of calibration curves when the uncertainties on the independent variables \( x_i \) (i.e. the concentrations of the calibration solutions) cannot be considered negligible with respect to those associated with the dependent variables \( y_i \) (i.e. the analytical response) and when correlation exists among \( x_i \) and among \( y_i \).

REFERENCES


Fig. 1: SERS spectra of 5 levels of melamine concentration with 40 nm AuNPs (melamine peak at 715 cm⁻¹) [1]

Fig. 2: INRIM method for the quantification of melamine by SERS

Fig. 3: Ishikawa diagram showing the contributions of the input quantities to the final uncertainty of \( R, a, b \).